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CHEMICAL COMPOSITION OF HUMAN SKIN SURFACE LIPIDS FROM BIRTH TO PUBERTY*

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ABSTRACT

Skin surface lipids from the foreheads of 51 subjects between the ages of five days and fifteen years were analyzed by quantitative thin-layer chromatography. The cholesterol concentrations were low at birth, at maximum levels at six years of age, and low again, almost to adult levels, at nine years. Conversely, average concentrations of wax esters were high at birth, low between the ages of three and six years, and rising towards adult levels by nine years. There was no significant change in the combined concentrations of triglycerides plus free fatty acids during development. Concentrations of squalene were highly variable, in contrast to the constant proportions previously reported for adults.

Several earlier investigations have revealed that the proportion of cholesterol in human skin surface lipids is much higher in children than in adults (1–4). This situation has been attributed to a high concentration of cholesterol in epidermal lipid, which becomes diluted with sebum containing little cholesterol, when sebaceous gland activity increases at the onset of puberty. In contrast, the squalene content of the skin surface lipids has been claimed to increase at puberty (2), indicating that this compound is a product of the sebaceous glands.

The development of a simple procedure for quantitative analysis of small lipid samples by thin-layer chromatography (5) has prompted us to reinvestigate the changes in skin lipids which occur between birth and puberty. This has revealed that in terms of skin lipid composition these are three distinct periods during development, and that the final change to a composition of the adult type begins several years before other recognizable signs of puberty.

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MATERIALS AND METHODS

Collection of lipids. Skin surface lipids were obtained by wiping the forehead with a small polyurethane sponge moistened with hexane. The collections were made at least 24 hours after the subjects had last washed, in order to obtain adequate quantities of material. The newborn children studied were five days old, and had been washed daily prior to collection of the surface lipid.

The lipids were recovered from the sponges by extraction with hexane and the solvent was evaporated under a stream of nitrogen. The lipid residues were stored at −20° C until analyzed.

Quantitative analysis. The principles governing the thin-layer chromatographic procedure have been described previously (5, 6). The lipid samples were taken up in 0.1 ml of hexane and suitable volumes of the solutions (usually 10 to 20 μl, containing between 5 and 10 μg of lipid) were spotted on thin-layer plates. These were standard 20 × 20 cm glass plates, coated with a 250 μ layer of Silica Gel G (E Merck & Co.) which had been ruled into 7 mm wide lanes. Prior to use the plates were developed in ether and activated by heating at 130° C for thirty minutes. After spotting, the plates were developed successively to 19 cm in hexane, to 19 cm in benzene, and finally to 10 cm in a mixture of hexane:ether:acetic acid (70:30:1). The plates were air-dried for ten minutes between each development.

The developed chromatograms were sprayed with 50% H_2SO_4 and charred by heating to 220° C over a period of thirty minutes. They were then quantitated by scanning with a photodensitometer (Photovolt Corp, model 530) as previously described (5, 6). Peak areas on the recorder charts were obtained by triangulation and the analyses were calculated from these areas after making al-

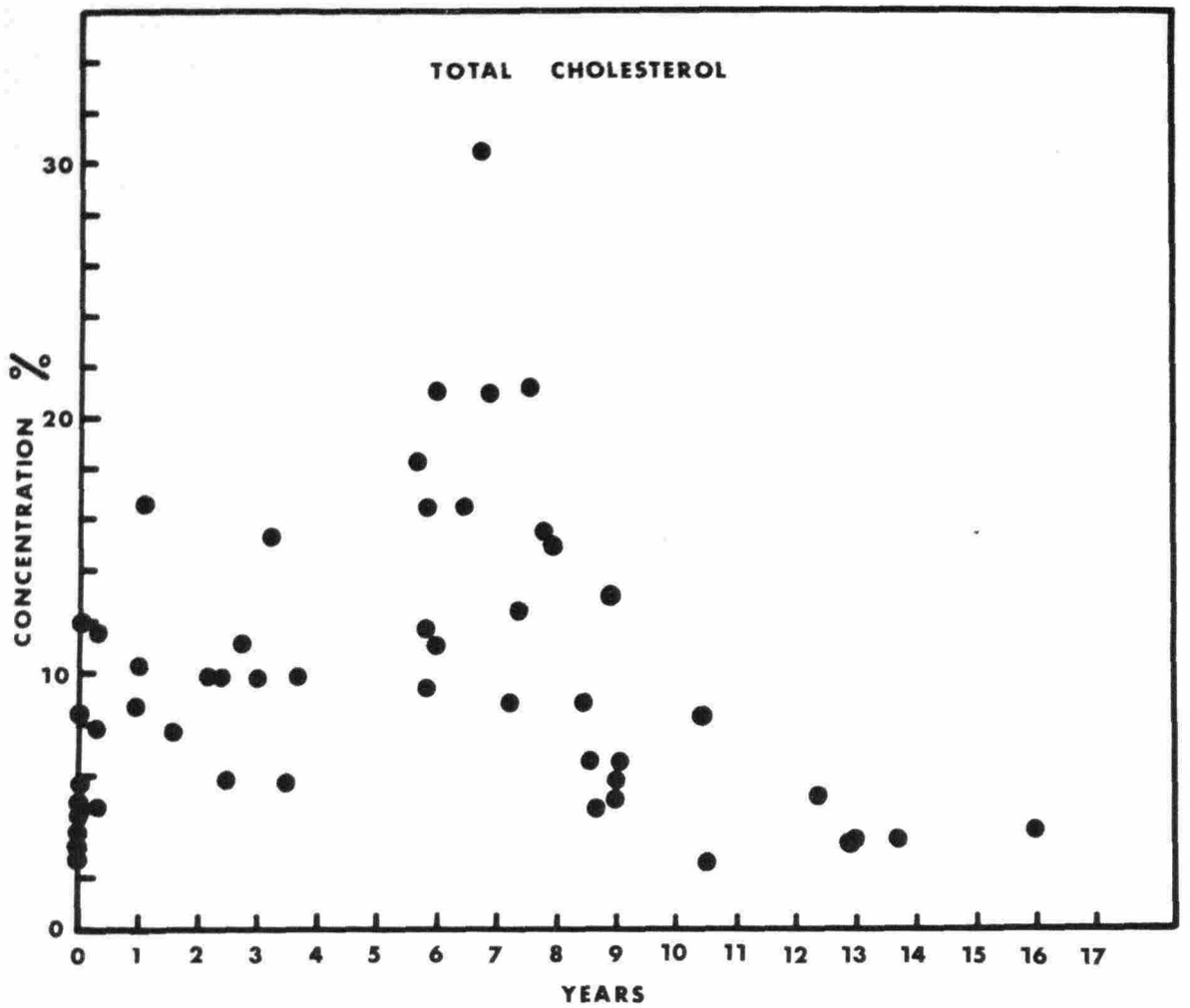

FIG. 1

lowance for the established efficiency of charring of the different lipids (5). This procedure has been shown to resolve and adequately quantitate all of the classes of lipids recognized as significant constituents of the skin surface film (5, 6).

It must be emphasized that only the composition of the recovered lipids was determined, and no attempt was made to measure the absolute amount of lipid obtained from each subject. Efficiency of recovery and the volumes applied on the chromatograms were therefore immaterial.

RESULTS

In Figures 1 through 5 the results of the individual analyses are plotted against the ages of the subjects. No distinction is drawn between males and females.

For Figure 1 the values for total cholesterol were calculated rather than separate figures for free and esterified cholesterol. This was done in order to eliminate variation resulting from differing degrees of conversion of free cholesterol to cholesterol esters under the influence of epidermal esterases. The results for total cholesterol were, nevertheless, highly variable up to nine years of age, but confirm the previously established tendency towards high values for cholesterol in the skin surface lipid of children. Our results differ in indicating that the cholesterol concentration begins to fall to adult levels several years earlier than previously believed.

In Figure 2 the proportions of wax esters in the surface lipid are plotted according to age of the subject. Here the reverse trend is apparent, with an increase in wax esters towards the adult level well-established by nine years of age. Again, the values were quite variable at earlier ages.

Some of the variability in the proportions of cholesterol and wax esters could conceivably have been due to differing degrees of topical contamination of the skin with food fats, which are almost exclusively triglycerides. The possible effect of this variable was eliminated by plotting the ratios of cholesterol to wax esters for each subject (Fig. 3). While this accentuated the magnitude of the relative changes in these components during development, the variability between one and nine years was not reduced and is, therefore, not due to contamination by other lipids.

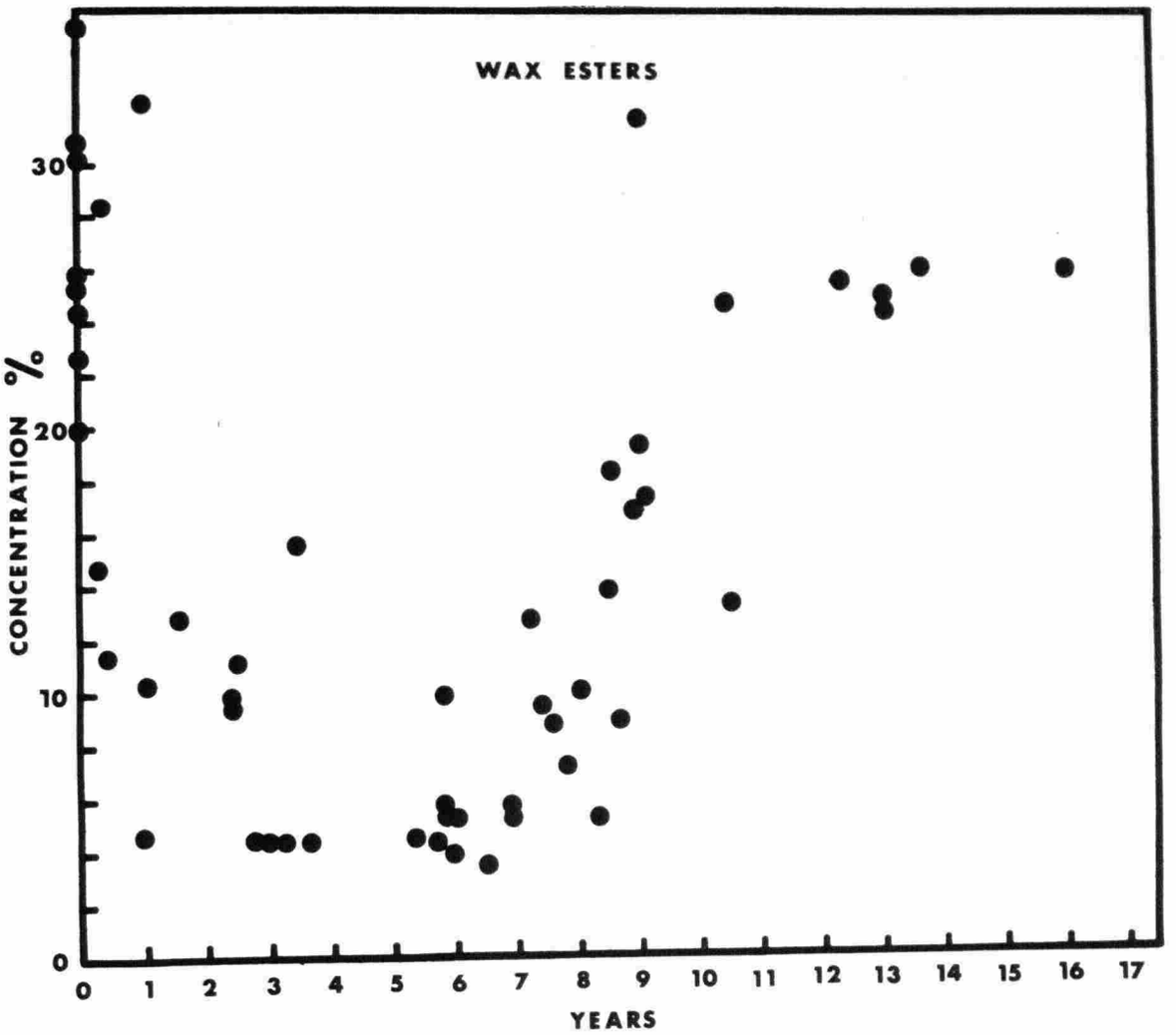
WAX ESTERS
CONCENTRATION %
0
10
20
30
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
YEARS

FIG. 2

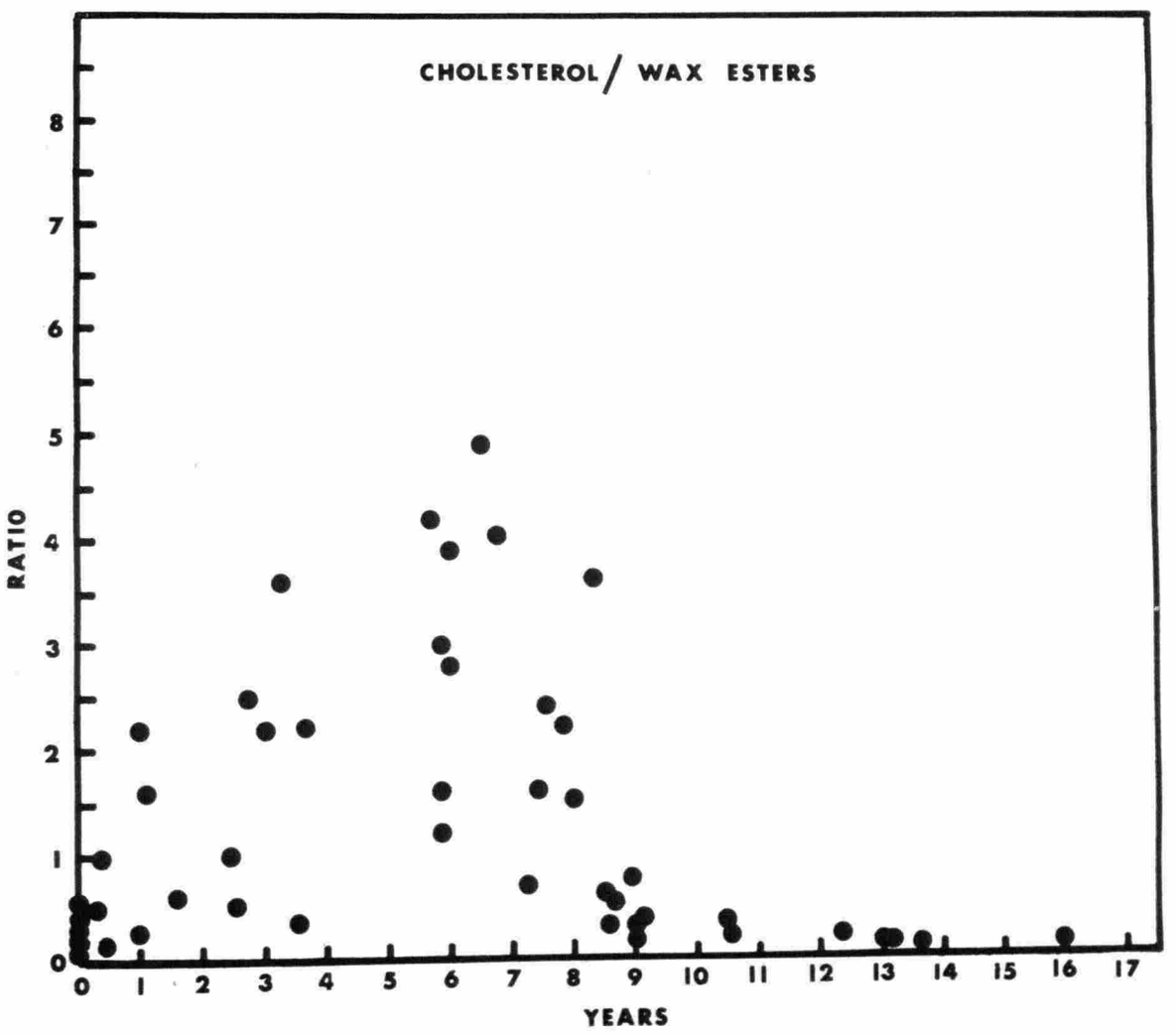
CHOLESTEROL / WAX ESTERS
RATIO
0
1
2
3
4
5
6
7
8
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
YEARS

FIG. 3

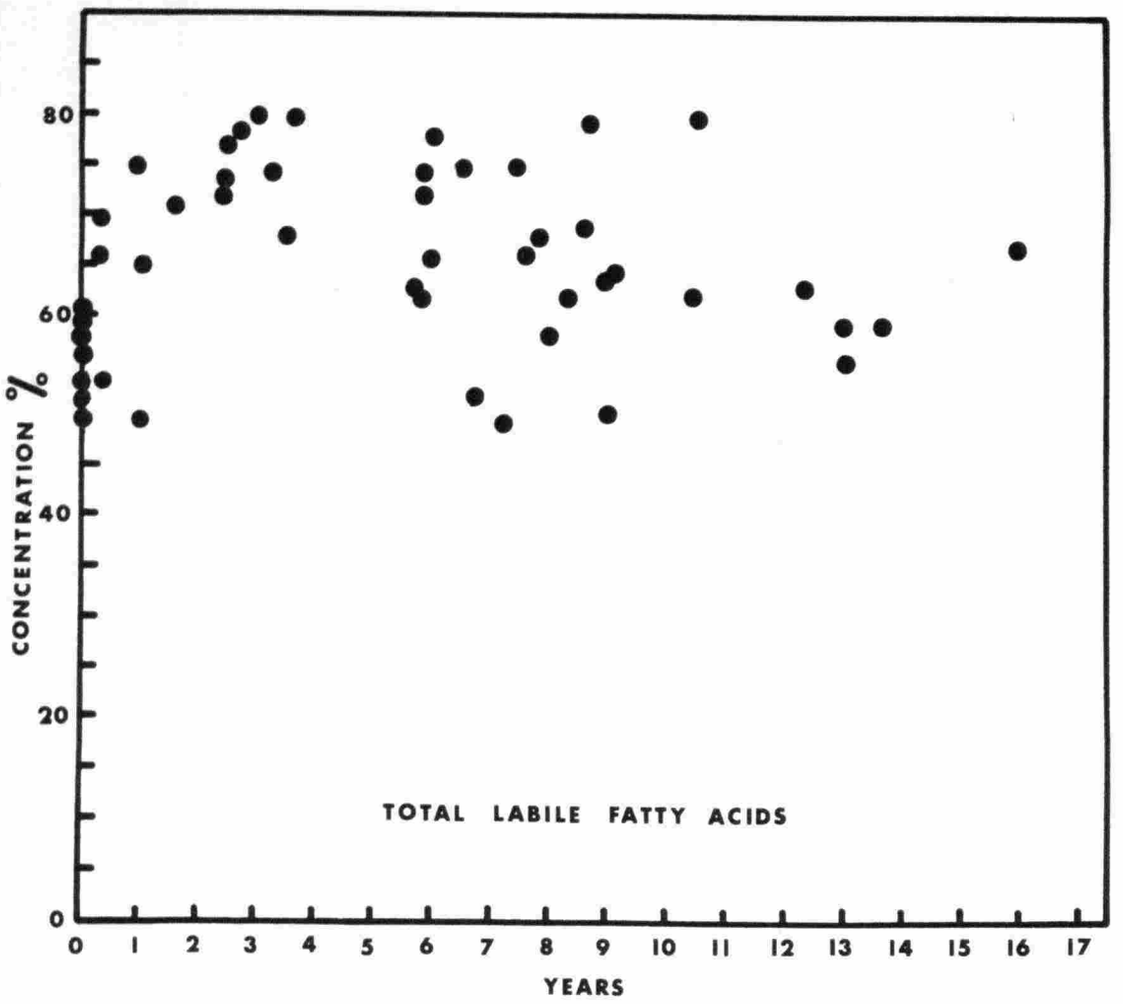
CONCENTRATION %
80
60
40
20
0
TOTAL LABILE FATTY ACIDS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
YEARS

FIG. 4

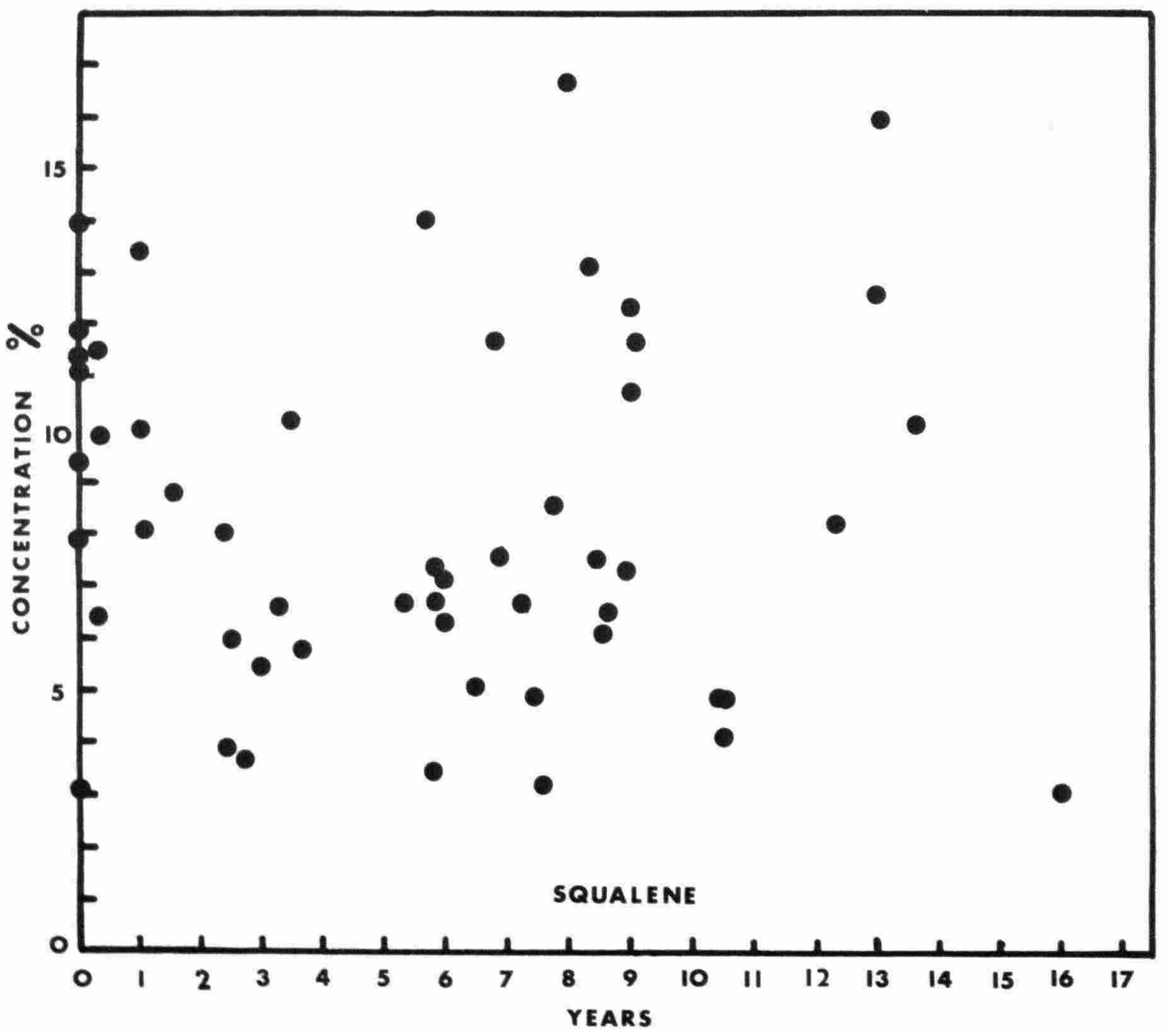
CONCENTRATION %
15
10
5
0
SQUALENE
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
YEARS

FIG. 5

TABLE I

Changes in surface lipid composition with age

Age		Subjects	Free fatty acids	Triglycerides	Wax esters	Cholesterol	Cholesterol esters	Squalene
Range	Avg. (mos)							
5 days	—	8	1.5	51.9	26.7	2.5	6.1	9.9
1 month–2 years	10	7	20.8	38.4	17.6	3.7	10.3	9.4
2–4 years	35	8	22.9	49.6	8.0	4.2	8.9	6.2
4–8 years	81	13	15.9	45.6	6.9	7.2	14.6	7.7
8–10 years	110	8	17.8	47.4	17.8	3.2	5.7	8.3
10–15 years	152	7	18.8	42.9	23.6	1.8	4.2	8.4
18–40 years	—	17	16.4	41.0	25.0	1.4	2.1	12.0

Considerable variation between individuals was apparent in the values for free fatty acids and triglycerides. Part of this variation was undoubtedly due to differences in the degree of hydrolysis of triglycerides by the action of bacteria. For the purposes of the present study this effect was minimized by calculating the sum of the figures for free fatty acids, triglycerides and diglycerides for each subject. This composite value is termed "total labile fatty acids." In Figure 4 the individual values for total labile acids are plotted according to the age of the subject, revealing no clearly-defined change during development, although there is a tendency to high values between one and five years.

Squalene was the most variable of the surface lipids studied, and no developmental trend was apparent (Fig. 5).

On the basis of the trends apparent in the proportions of cholesterol and wax esters, the average surface lipid compositions were calculated for a series of age groups (Table I). For comparison, an average composition of adult surface lipid (6) is included in the table. The highest concentrations of cholesterol and cholesterol esters and the lowest proportion of wax esters occur in the range four to eight years, after which the composition changes towards adult values.

DISCUSSION

The average surface lipid compositions for the different age groups shown in Table I confirm the observation of earlier investigators that cholesterol content is high in prepuberal children. This is in agreement with the accepted view that since sebum production is low in this age group, the cholesterol-rich epidermal lipid is predominant. However, the age at which the change toward an adult-type composition occurs is earlier than expected from previous observations, which have suggested that the cholesterol concentration decreases at about 13 to 15 years. Thus, Nicolaides and Rothman used pooled hair lipid from boys aged six to twelve years to represent child sebum, which was found to contain 12.2% total cholesterol, compared with 3.8% for men (2). Washburn and Liese reported an average 8.7% cholesterol for children aged three to sixteen years (3). Our data indicates that the change to adult-type composition of the surface lipids is well advanced by age nine.

There is also a marked change in surface lipid composition between birth, where it resembles the adult composition, and four years of age, indicating that sebum production is high in the newly born. Presumably the sebaceous glands are active at birth as the result of stimulation by maternal androgens acquired transplacentally.

If a change in the ratio of sebum to epidermal lipid in the surface film is accepted as the sole mechanism for the change in composition with development, the analytical figures may be used to indicate the anatomical origin of other constituents. Thus, the dramatic increase in wax ester concentration towards puberty is evidence for the origin of this material in the sebaceous glands. This agrees with Nicolaides' observation of minor amounts of wax esters in plantar epidermis (7). Similar interpretation for triglycerides plus free fatty acids, which together show no appreciable change with age suggests that this fraction forms a similar proportion of both sebum and epidermal lipid. It is of interest to note, how-

ever, that at the age of five days there is almost no hydrolysis of triglycerides to free fatty acids, suggesting that a population of the appropriate bacteria has yet to be established.

From the average values in Table I, it would also be concluded that squalene is present in both sebum and epidermal lipid. In this regard, Nicolaides and Rothman demonstrated some synthesis of squalene by palmar epidermis (8), although only traces were detected in plantar epidermis (7). However, the concentrations of squalene are too variable to form a basis for interpretation at this time.

It is of interest to inquire into the large differences between individual children in the composition of the skin surface lipids. This variation would not appear to result from deficiencies in the analytical procedure, judging from the results of repetitive analysis of reference mixtures and of samples from individual subjects (5, 6). The most probable source of this variation would appear to be large differences in the ages at which sebum production changes, both post-natally and pre-puberally. Thus, in a particular age group, some children may have accomplished a change while others have yet to experience this effect. In order to examine this aspect we are presently following the skin surface lipid composition of individual children over long periods of time.

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